Activity increases quantal size at the frog neuromuscular junction[1]

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Summary. Two h of nerve stimulation at 10 Hz or of elevated spontaneous release in hypertonic solution increased the size of miniature end-plate potentials (m.e.p.p.'s) and currents at the frog neuromuscular junction, probably by increasing the amount of acetylcholine in a quantum. Increases in quantal size may modulate synaptic transmission.
Key words. Rana pipiens; frog muscle; neuromuscular junction; spontaneous hypertonic release; nerve stimulation; miniature endplate potentials, acetylcholine quantum.

The experiments were performed on *Rana pipiens*, which we routinely store in a darkened room at 10 °C. We stimulated the nerve tetanically in vivo to maintain the oxygenation of the muscle. The brain and spinal cord were pithed. The nerves running to the leg on one side were cut; those on the other side were stimulated. Oxygen flowed over the animal. The nerve was stimulated for 2 h at 10 Hz. Then both sartorius muscles were dissected and placed in Ringer. Within 15 min after the end of stimulation microelectrode recording of m.e.p.p.'s from an end-plate was begun. In some experiments the stimulated end-plate was examined first and vice versa. The junctions from the stimulated muscle showed an unusually high frequency of large m.e.p.p.'s (fig. 1A). The m.e.p.p.'s were digitized and stored on magnetic discs. Later each was displayed on a computer terminal; records containing overlapping m.e.p.p.'s were rejected. The amplitudes and areas of the m.e.p.p.'s were measured. Usually 100 m.e.p.p.'s were recorded from each of 5 end-plates. Then the results from each muscle were merged and plotted as a cumulative frequency distribution. Both the median amplitudes (fig. 1B) and the median areas of the m.e.p.p.'s (fig. 1C) from the stimulated muscles were more than twice those from the resting muscle. Similar results were obtained in 6 additional experiments with the same protocol. The miniature end-plate currents (m.e.p.c.'s), measured with the 2 electrode voltage clamp, from these preparations were also larger. This demonstrates that the increased size cannot be accounted for by an increase in the input resistance of the muscle fiber.

Other preparations were stimulated to release large numbers of quanta by exposure to hypertonic solutions followed by a return to normal Ringer. The stimulation by this method caused an increase in m.e.p.p. and m.e.p.c. size similar to that caused by nerve stimulation.

Replacing the chloride in the hypertonic Ringer with isothionate[2,3] or gluconate produced a further substantial increase in quantal size after the preparation was returned to normal Ringer (fig. 2A and B). Many of the large m.e.p.c.'c recorded after this treatment often have a slow rise (fig. 2A), which is why we prefer to use areas in our measurements. Recordings with extracellular microelectrodes showed that both slow and fast rising m.e.p.p.'s were recorded at each placement, which suggests that both are generated at the same points along the terminal. There was less of an increase in m.e.p.p. size when the Ringer contained thiocyanate in place of chloride. Thiocyanate inhibits the active transport of acetylcholine (ACh) into synaptic vesicles[4]. Perhaps the anions act by affecting the loading of the vesicles.

Vrbova' and Wareham[5] reported a 1.3-fold increase in m.e.p.p. amplitude following prolonged nerve stimulation in the rat. They also found that the cation of ACh in the bath produced an increased depolarization of the stimulated muscles, showing that the ACh sensitivity of the end-plate is increased. We tested the effects of 2×10^{-6} g/ml ACh in Ringer containing 10^{-6} g/ml of neostigmine Br on muscles pretreated for 2 h in 200 mM Na gluconate Ringer and on paired muscles pretreated in 120 mM NaCl Ringer. We used this treatment because it produces the largest changes in quantal size. There was no significant difference in the depolarizations produced (gluconate: 19.6 ± 1.1 mV, $n = 13$; Cl: 17.8 ± 1.1 mV, $n = 9$). The relatively small increase in ACh sensitivity found in the rat may also occur in the frog, but not be detected because of a greater scatter in the data; it could not account for the increa-

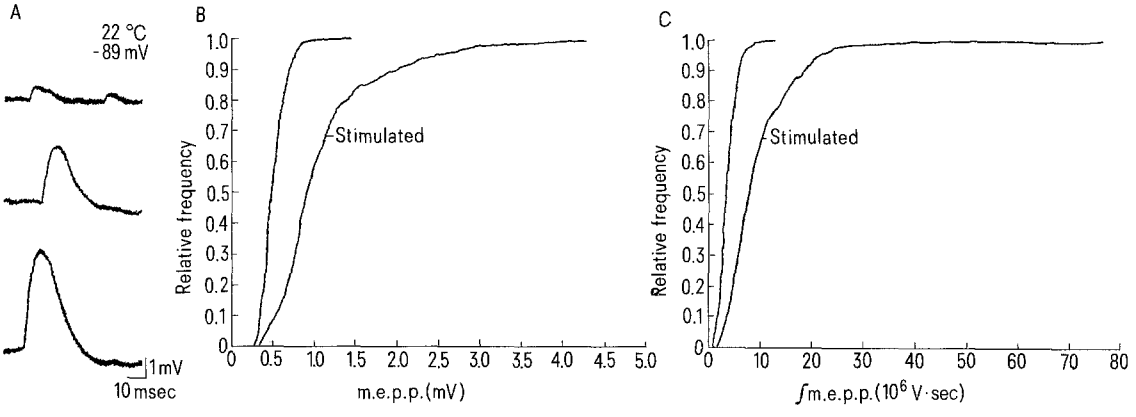

Figure 1. *A* Photographs of m.e.p.p.'s recorded from an unstimulated muscle (upper panel) and from the contralateral, stimulated muscle (middle and lower panel) of the same frog. The m.e.p.p.'s shown from the stimulated muscle were among the largest seen. *B* Cumulative frequency distributions of m.e.p.p. amplitudes from the unstimulated muscle (left) and from the stimulated muscle (right). The median is roughly doubled following the stimulation and there are some m.e.p.p.'s of high amplitude. *C* Cumulative frequency distributions of m.e.p.p. areas recorded from the unstimulated (right) and stimulated (left) muscles. The areas are proportional to the total charge that entered the end-plate as a result of the action of the transmitter. The Ringer contained (in mM) 120 NaCl, 2.0 KCl, 2.5 $CaCl_2$, 4.0 N-tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid (TES) at pH = 7.4 and 5×10^{-4} tetrodotoxin. The probability that the difference could occur by chance is < 0.001 by the Kolmorogov-Smirnov test[7]. M.e.p.p. amplitudes were corrected to a resting potential of −90 mV[8].

ses in quantal size we observed. Noise analysis of the end-plate response to ACh[6] showed that stimulation did not produce detectable changes in the single channel lifetimes or conductances (W. Van der Kloot, in preparation).

We measured the size of quanta released by nerve stimulation in high Mg^{2+}, low Ca^{2+} Ringer, in which the probability of a quantum being released by the stimulus is less than one. In preparations pretreated for 2 h in hypertonic NaCl Ringer, the size of the quanta released spontaneously and following stimulation were of similar sizes. In preparations pretreated with hypertonic gluconate Ringer, some of the largest quanta may only be released spontaneously.

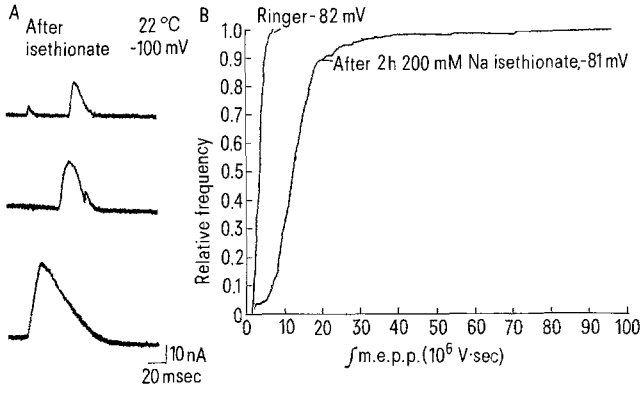

Figure 2. *A* A sartorius muscle was soaked for 2 h in a Ringer containing 200 mM Na isethionate (replacing the 120 mM NaCl). Then it was returned to normal Ringer and voltage clamped at −100 mV. The upper trace shows one of the smaller currents. The lower trace shows one of the largest. Note the slow rise of the large current. The amplifiers were AC coupled, which is responsible for the undershoot of the base line following the huge m.e.p.c. *B* In another experiment a microelectrode was inserted at an end-plate and the areas of 100 m.e.p.p.'s were measured (left). Then the preparation was transferred to Ringer containing 200 mM Na isethionate for 2 h. After the return to Ringer the areas of 100 m.e.p.p.'s were measured. The electrode remained in the fiber throughout the experiment; the resting potential decreased by 1 mV.

All of the treatments that elicit an increase in quantal size involve a period in which many quanta are released from the nerve terminal. We speculate that following quantal release at a high rate subsequent quanta contain an increased amount of ACh. An obvious hypothesis is that stimulation increases the size or the ACh content of synaptic vesicles. Anions may affect the quantal size by altering the uptake of ACh into vesicles. Obviously a mechanism that changes quantal size following activity could play a role in the physiology of the neuromuscular junction. To demonstrate the effect unequivocally we stimulated for long periods, and the changes were several-fold. We have found that stimulation for 1 h still produces large increases in quantal size, but have not yet studied shorter periods of stimulation. Much smaller changes in quantal size could be physiologically significant at other synapes. If a similar mechanism is found at central synapses it might play a role in the modification of behavior by experience.

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Influence of diet on plasma tryptophan and brain serotonin levels in mice

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Summary. Groups of mice were maintained for up to 78 weeks on tryptophan restricted, protein restricted and control diets. Plasma tryptophan levels were significantly reduced by both forms of dietary restriction. Brain serotonin levels were significantly reduced only in mice on the tryptophan restricted diet, but not for mice on the protein restricted diet. The protein-restricted diet contains less of the large neutral amino acids which compete with tryptophan to enter the brain. It is known that protein restriction and tryptophan restriction extend lifespan. The results presented here suggest that extension of lifespan and lowering of brain serotonin are not related.
Key words. Mice, brain; mice, plasma; brain, mouse; plasma, mouse; serotonin, brain; tryptophan, plasma; brain serotonin; dietary restriction.

Dietary restriction is the most effective means of extending the lifespan of rodents[1-3]. Several methods of restriction have produced significant life-extension. These include reduction of total food intake, reduction of protein intake, and reduction of tryptophan intake. For example, mice on a low (4%) protein diet live 39% longer than control mice on a 26% protein diet[4]. Similarly rats on a low tryptophan diet live 20% longer than controls[5,6]. Based on these observations, the question arises as to whether low protein and low tryptophan diets produce life span extention by similar biological mechanisms.